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USES, PREPARATION, AND PROPERTIES OF PINGUINAIN, THE PROTEIN-SPLITTING ENZYME OF THE *Maya* FRUIT. *By C. F. ASENJO and M. DEL C. C. DE* FERNÁNDEZ 1

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USES, PREPARATION, AND PROPERTIES OF PINGUINAIN, THE PROTEIN-SPLITTING ENZYME OF THE *MAYA* FRUIT

By C. F. ASENJO and M. del C. C. de FERNÁNDEZ

From the Department of Chemistry of the School of Tropical Medicine and the Agricultural Experiment Station of the University of Puerto Rico

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The existence of a protein-splitting enzyme in *maya* (*Bromelia pinguin* L.) was first established by Asenjo and Fernández in 1941 (1) and the name "pinguinain" suggested for this new enzyme. Pinguinain belongs to that group of enzymes known as papainases, that is, enzymes similar to papain which clots milk and digests proteins and live tissue. The milk-clotting activity of pinguinain is similar in many respects to that of the renin enzyme. Preliminary experiments carried out on it suggest its use as a substitute for this animal enzyme in the manufacture of cheese.

The above-mentioned properties of the papainases have already been put to practical usage both in industry and in medicine. Every year, beer manufacturers utilize a large amount of papainases in the preparation of "chill-proof" beers. When chilled, some beers become turbid because of small particles of protein and other associated substances that precipitate during the chilling process; when beers are pretreated with a proteainase (Wallerization), such as papain or pinguinain, such particles are digested and a perfectly clear beer is produced at low temperatures. Pinguinain

has already been tried by several consulting laboratories who report that it has proved very satisfactory for the above purpose.

The papainases are also abundantly used in the meat-packing industry, therefore another practical usage of pinguinain would be as a meat-tendering agent. Pinguinain has been found to soften meats readily and could compete favorably with papain and bromelin—the two enzymes now currently used as tenderizers—if produced on a commercial scale.

Many pounds of protein-splitting enzymes are used yearly in the tanning industry, since raw hides have to be treated with a proteinase to make them softer and more porous before being submitted to the action of the tanning reagent.

Lastly, the authors wish to call attention to the worm-digesting activity of pinguinain. Experiments have shown that *maya* juice, or the crude enzyme obtained from it, readily digests live intestinal parasites *in vitro* and *in vivo*, in experimental animals. For a long time now the juice of the *maya* fruit has been utilized as an anthelmintic in the folk medicine of the West Indies.

These potential applications of pinguinain in the practical field suggest the desirability of developing its production; so towards this end the Department of Agronomy of the Agricultural Experiment Station at Río Piedras, Puerto Rico, is studying the methods of planting and harvesting the *maya* fruit on a commercial scale.

In the present communication the authors report several observations dealing with the preparation and properties of pinguinain.

EXPERIMENTAL

The maya plant. This plant (Fig. 1) belongs to the pineapple family and has been described by Britton (2) as follows:

"*Bromelia pinguin* L.: Leaves many, tufted stiff, linear long-attenuate, 1–2 m. long, 2–4 cm. wide, light green, the margins armed with stout, rather distant hooked prickles 5–10 mm. long. Inflorescence paniculate, shorter than the leaves, stout, densely white-floccose; bractlets narrow, 5–25 mm. long; sepals narrow, erect, triangular-subulate; petals white or pinkish, about 3 cm. long, linear-elliptic, united below, the apex white-tomentose; stamens about 2 cm. long; anthers yellow, linear, 1–1.4 cm. long; ovary white-farinose, subterete. Berry ovoid, yellow, beaked, verruculose, 3–4 cm. long.

Thickets, hedges, and waste grounds, Porto Rico; St. Thomas, St. John, St. Croix: —West Indies and continental tropical America. Piñuela, Maya, Pinguin."

The plant is extensively utilized in the rural districts of Puerto Rico as a natural barrier or boundary. Attempts were made in the past to use its leaf fibers industrially.

The Maya fruit. The *maya* fruit grows in the center of the plant in the form of a bunch, as shown in Fig. 2, consisting generally of about 63 per cent fruit and the rest stalk. The fruit is available in appreciable amounts between May and November, but is difficult to obtain during the remainder of the year.

Two varieties of *maya* have been observed: a long, thin one that gives a poor yield of juice and a round, thick one, shown in Fig. 2, which yields a goodly amount. The first variety is found in sandy soils near the seashore,

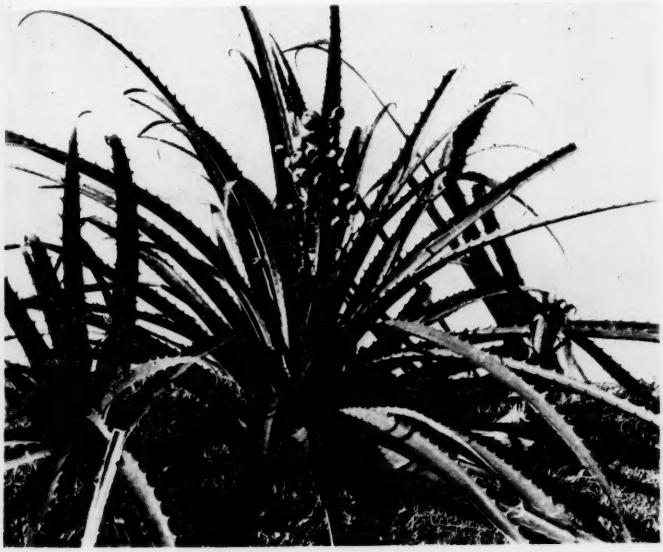

FIG. 1. *Maya* plant with fruit stalk

while the latter grows in the interior of the Island at higher elevations. The authors have primarily utilized the last variety in their work.

Expression of the juice. The juice is prepared by squeezing the pulp in a cheesecloth as follows: the fruit is cut in four pieces; the pulp removed and pressed until dry of juice. The expressed juice is somewhat viscose but, left overnight in the icebox, the sludge settles to the bottom of the vessel and the supernatant fluid may be syphoned and used in the preparation of the enzyme. Attempts to centrifuge out the viscose sludge have proved unsuccessful; it may be removed only as described above, that is, by slow settling in the icebox. It is very important to remove this substance, which is primarily present in the rind of the fruit, before precipitating the enzyme; otherwise the latter is rendered sticky and difficult to dry. The author's

attempt to press the whole fruit produced an exceedingly viscose juice which could not be used in the preparation of the enzyme.

The pH of 10 different samples of *maya* juice fluctuated between 3.70 and 4.30 with an average of 3.75.

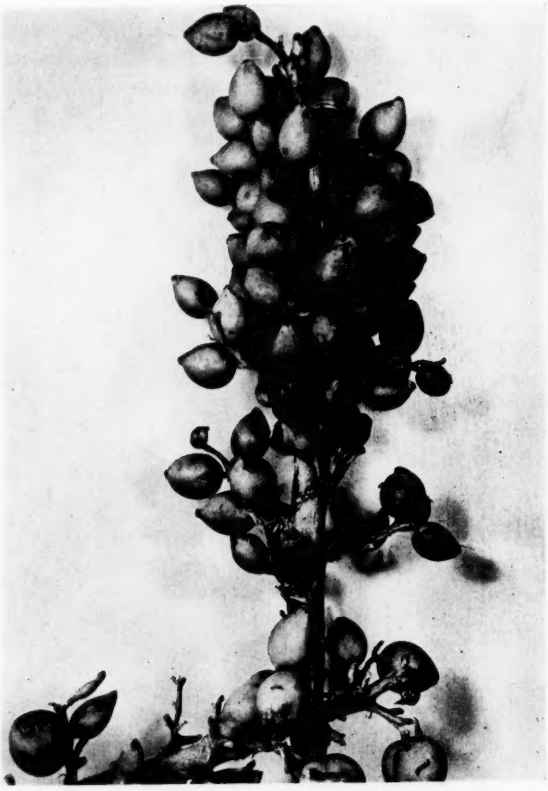

FIG. 2. Close view of fruit raceme

The activity of this juice varies somewhat. Such variation is recorded in table I for three different batches of juice in terms of milk-clotting units[1] and formol titrations (ml. 0.01N NaOH per ml. of juice).[2]

[1] The milk-clotting method of Ball and Hoover (3) was used throughout this work. Only 5 ml. of milk solution per tube were used hence, the milk-clotting units obtained were 0.546 times smaller than those resulting from the use of 10 ml. of milk.

[2] Throughout these studies, the technique utilized in formol titration was as follows: 2 per cent gelatin solution containing 1 per cent toluene, as preservative, was

Preservation of the juice. During the time that the authors have been working with *maya* juice, they observed that its activity diminished rapidly at room temperature, produced primarily by fermentation that set in from four to five hours after the juice was expressed. However, icebox temperatures of 4 to 8°C. preserved the juice for 24 to 48 hours without appreciable loss of activity, after which the activity again began to diminish rapidly.

TABLE I

Proteolytic activity of maya juice

Batch	Milk-clotting units per ml. of *maya* juice	Formol titration ml. 0.01N NaOH per ml. of *maya* juice
1	50.0	3.80
2	45.0	3.70
3	47.6	4.03
Average.....	47.5	3.84

TABLE II

Preservative action of different substances on the milk-clotting activity of maya juice at room temperature (25–28°C.)

No. of days standing at room temperature	Percentage of the original activity lost					
	Thymol 90 mg./100 ml.	Sodium bisulphite 30 mg./100 ml.	Toluene 90 mg./100 ml.	50–50 toluene chloroform mixture 90 mg./100 ml.	Sodium benzoate 90 mg./100 ml.	Merthiolate 30 mg./100 ml.
1	25	38	46	33	52	4
2	50	52	55	54	65	4
6						4
12						4
29						46

Several reagents were tried as preservatives and their effect on the proteolytic activity of the juice was measured by means of the milk-clotting assay at different time intervals (Table II). Merthiolate (Sodium Ethyl Mercuri Thiosalicylate) was the only reagent that gave satisfactory results. Thirty milligrams kept 100 ml. of juice at room temperature for 12 days

used as a substrate. One ml. of the juice to be tested was then added to 10 ml. of this gelatin solution and one ml. samples taken for titration. The indicator was a solution containing 0.2 per cent phenolthalein in 50 per cent alcohol. One ml. of 40 per cent formaldehyde was then added to each volume of mixture to be titrated; the strength of the sodium hydroxide solution was 0.01N. These tests were run for a period of 24 hours at an incubation temperature of 40°C.; no buffer was used. The natural pH of the mixture was found to be in the neighborhood of 4.5 to 5.

with only a slight loss of activity. However, after 29 days, the percentage loss of the original activity was 46 per cent. The authors used merthiolate at the above concentration all through the work to preserve juices and enzyme solutions which were kept for a few days with very good results.

TABLE III

Percentage yield of pulp, juice and enzyme preparation by weight of fresh fruit

Fruit batch no.	Percentage of pulp by weight of fresh fruit	Percentage of juice by weight of fresh fruit	Percentage enzyme yield by weight of fresh fruit
1	57.0	29.5	0.5
2	59.9	30.5	1.0
3	59.5	21.6	0.4
4	65.0	36.4	2.3
5	66.6	32.3	1.7
Average	61.6	30.1	1.1

TABLE IV

Proximate composition of crude pinguinain

Total moisture per cent	6.0
Reducing sugars per cent	11.0
Nitrogen per cent	2.7
Substances soluble in ether per cent	1.1
Ash per cent	10.2

TABLE V

Milk-clotting activity of pinguinain

Batch No.	Milk-Clotting Units Per Gram
1	434.7
2	500.0
3	526.8
4	416.7
Average	469.5

Preparation of the enzyme. The crude enzyme was prepared by adding three volumes of acetone to one of juice, the precipitate obtained thereby separated by centrifugation, washed with fresh acetone and diethyl ether, and dried in a vacuum desiccator over $CaCl_2$.[3] The yield of enzyme for

[3] In the original communication (1) the authors recommended dissolving this precipitate in 0.02 M NaCN and reprecipitating it with acetone. Later on they found that this step could be eliminated without affecting the quality of the final product.

several batches of fruit is recorded in Table II and averaged 1.1 gm. per 100 gm. of fruit, or 3.7 gm. per 100 gm. of juice.

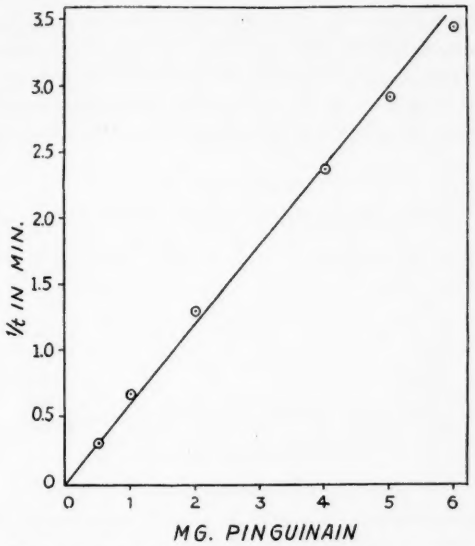

FIG. 3. Milk-clotting activity of pinguinain dissolved in 0.05 M NaCN

TABLE VI

Milk-clotting by pinguinain in 0.05M NaCN

Amount of Enzyme Per 6 ml. (E)	Time of Coagulation (t)	1/t	E.t = K
mg.	*min.*		
6.0	0.29	3.45	1.7
5.0	0.34	2.94	1.7
4.0	0.42	2.38	1.7
2.0	0.77	1.32	1.5
1.0	1.50	0.67	1.5
0.5	3.42	0.30	1.7
Average			1.6

General characteristics of the crude pinguinain. Generally, pinguinain has a very light greenish yellow color. It is an amorphous powder that dissolves somewhat slowly and produces an opalescent solution. This enzyme readily reduced Fehling's solution and gave a positive Millon's reaction for proteins. Its proximate composition is shown in Table IV.

Activity of pinguinain. The milk-clotting activity of four different batches of pinguinain are recorded in Table V, with an average activity of 469.5 milk-clotting units per gram.

The milk-clotting activity of various dilutions of pinguinain were determined by using 0.05M NaCN as diluent and the results obtained plotted in Fig. 3. The reciprocal of the milk-clotting time varied linearly between 0 and 6 mg. of enzyme. As in the case of crystalline chymotrypsin (3), apparently none of the enzyme was inactivated, as the curve passed through

TABLE VII

Digestion of casein in water by different amounts of pinguinain

Enzyme in 6 ml. digestion mixture	N. P. N. in 6 ml. digestion mixture 20 min. digestion
mg.	*m-eq.*
10.0	0.330
5.0	0.249
1.0	0.044
0.5	0.032
0.1	0.014

TABLE VIII

Digestion of hemoglobin in urea at 40°C. by pinguinain dissolved in 0.05M NaCN

Enzyme in 6 ml. of digestion mixture	Tyrosine in 6 ml. digestion mixture after 10 minutes
mg.	*m.-eq.* $\times 10^3$
10.0	6.37
5.0	4.90
3.0	3.6
1.0	1.82
0.5	1.21
0.1	0.19

the origin; therefore, the simple equation $E \cdot t = K$ held for this range of dilutions.

This particular batch of pinguinain, when dissolved in 0.05M NaCN, had an activity of 606 milk-clotting units per gram, as computed from table VI.

The proteolytic activity of pinguinain on casein and hemoglobin was measured by the Northrop (5) and the Anson (6) methods, respectively; the results of these determinations are given in Tables VII and VIII.

The quantity of casein digested, in m-eq., was plotted against the quantity of enzyme (Fig. 4); the variation was approximately linear up to 5 mg. of enzyme. From a straight line drawn tangent to the initial part of this

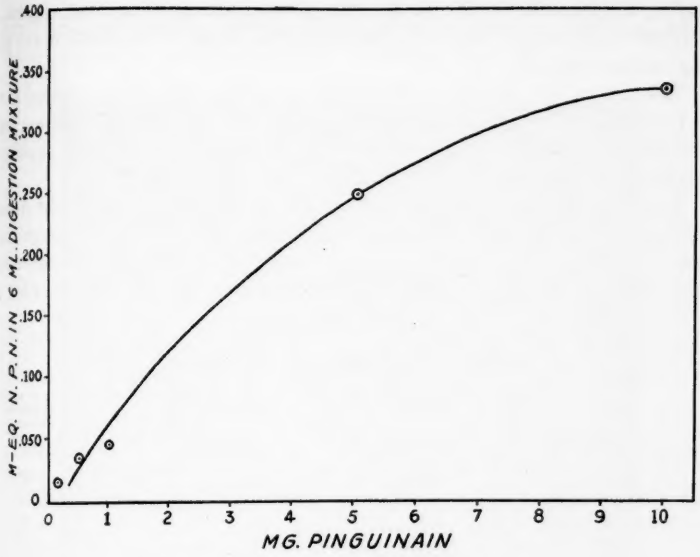

FIG. 4. Digestion of casein by pinguinain

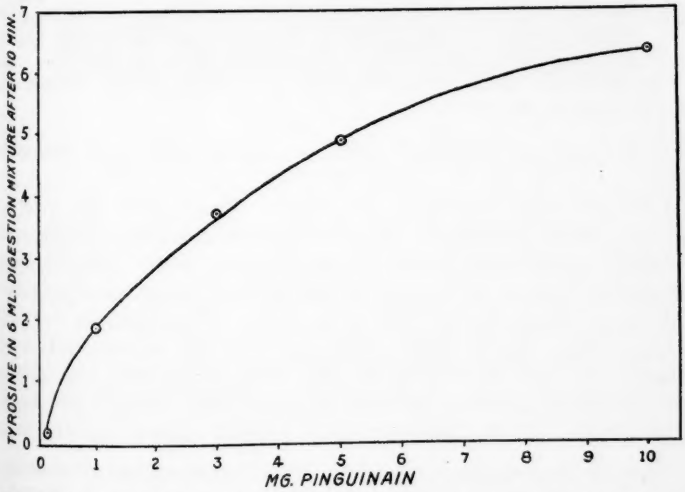

FIG. 5. Digestion of hemoglobin in urea by pinguinain dissolved in 0.05 M NaCN. Tyrosine is in m.-eq. $\times 10^3$

curve, the quantity of N. P. N. (non-protein nitrogen) in 6 ml. digestion mixture, yielded by 1 mg. of pinguinain, was found to be 0.055 m-eq. per mg. of the enzyme.

The proteolytic activity of pinguinain on hemoglobin was evaluated from a plot of tyrosine color against weight of enzyme. The pinguinain was dissolved in 0.05M NaCN to prevent its inactivation by the oxidative action of hemoglobin.

By drawing a line tangent to the first part of the curve, (Fig. 5) the Anson protease unit[4] was calculated. It was found that 0.4 mg. of pinguinain gave a color value of 0.001 m.-eq. of tyrosine in 6 ml. digestion mixture after ten minutes. One Anson unit was, therefore, contained in 0.4 × 1000 ×

TABLE IX

Activation and inhibition of pinguinain

Reagents added to enzyme solution		Reciprocal of the milk-clotting time in min. 4 mg. enzyme	Ratio of activity to that of untreated enzyme
Inhibitor	Activator		
None*	None	1.560	1.00
0.00014M I_2	None	0.189	0.12
0.00014M I_2	0.2M NaCN	1.190	0.76
0.00028M I_2	None	0.086	0.06
0.00028M I_2	0.2M NaCN	1.062	0.68
0.00014M I_2	None	0.183	0.12
0.00014M I_2	0.3M cysteine HCl	1.390	0.89
0.00014M I_2 and boiled	0.3M cysteine HCl	0.000	0.00
None	H_2S bubbled for 3 min.	2.130	1.36

* The untreated enzyme solution contained 4 mg. enzyme per ml. The pH of the untreated solution was 5.9.

10 = 4000 mg., or one gram of enzyme contained 0.25 Anson hemoglobin units.

Activation and inhibition of pinguinain. Pinguinain, like other papainases, responds readily to the activating action of some reducing agents and also to the inhibiting action of some oxidizing ones. Table IX records the action of some of these agents on the milk-clotting action of pinguinain.

Action of pinguinain on intestinal parasites. *Maya* juice, as well as the crude enzyme, readily digests intestinal parasites *in vitro* and *in vivo*. Dog *Ascaris* and *Macracanthorhynchus hirudinaceus* from hog intestines were incubated at 40°C. with *maya* juice and with a one per cent solution of pinguinain. These parasites showed definite signs of digestion in two

[4] Anson defines one protease hemoglobin unit as the amount that will digest hemoglobin under standard conditions at an initial rate so that there is liberated per minute an amount of split products not precipitated by trichloroacetic acid which will give the same color with phenol reagent as one milliequivalent of tyrosine.

hours and were totally disintegrated in ten. Controls, in solution, inactivated by previous heating to 100°C., were not digested.

FIG. 6. *Ascarids* digested *in vivo* by pinguinain

TABLE X

Action of maya juice and pinguinain on dog ascaris in vivo

Dog No.	Dog Weight in kg.	Dose Administered	Egg Count		Number of partly digested ascarids in stools	Autopsy Findings
			Before Treatment	After Treatment		
1	2.3	10 gms. Pinguinain	19,972	3,233	5	
1	2.3	200 ml. *maya* juice	3,233	negative	1	No ascarids found in the intestine
2	3.5	200 ml. *maya* juice	5,167	negative	4	No ascarids found in the intestine

Animal experiments were carried out on dogs infected with *Ascaris*. On administering both the juice and the enzyme, by the use of stomach tube, the ascarids were expelled in the feces in a partially digested condition (Fig. 6). The details of this experiment are reported in Table X.

Both the fresh *maya* juice and the pinguinain in the dosages given above readily digest dog *Ascaris in vivo*.

SUMMARY

Some of the uses, methods of preparation, and properties of pinguinain, the protein-splitting enzyme of the *maya* fruit, have been considered and studied herein.

RESUMEN

La pinguinaina es un enzimo proteolítico del grupo de las papainasas, la cual se haya en el zumo de la fruta de la maya (*Bromelia pinguin* L.). En el presente artículo se describen sus posibles usos medicamentosos e industriales y se estudia su preparación, conservación y acción proteolítica sobre varios substratos.

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EFFECT OF LIME AND PHOSPHORUS ON THE YIELD OF FOUR LEGUMINOSAE IN TWO ACID SOILS OF PUERTO RICO

By J. A. BONNET, E. A. TELFORD, F. MARIOTA and P. TIRADO SULSONA[1]

INTRODUCTION

Conservation of soil nitrogen and organic matter is of paramount importance in the tropics. Although some Leguminosae grow well in the acid soils of the humid region of Puerto Rico it is believed that maximum yields of green manuring and maximum nitrogen fixation have not been obtained, in Puerto Rico, because little attention has been given to some practices which have a direct bearing on optimum growth of these plants. Among these practices, the application of lime to acid soils and the application of phosphorus to soils deficient in it are of importance.

EXPERIMENTAL WORK

Two one-acre fields of the soil type "Fajardo clay", at the Experiment Station farm in Río Piedras; and two of the soil type "Catalina clay" level phase, at "Las Ochenta" farm in Mayaguez, were chosen for the site of the experiment. Two fields were chosen at each place to study also the effect on the crop yield of sweet potatoes of two methods of incorporating the green manure. In field "A", the green manure was turned-under with a hoe as done by small farmers. In field "B", it was plowed under with a tractor-driven plow as done by the large landowners.

"Fajardo clay" is an acid soil of the humid region derived from old, high alluvial material and from outwash fans of adjacent shale hills. The relief is level or gently sloping. In a cultivated field it has a granular-brown or reddish-brown, friable, clay surface about 9 inches thick, fairly easily penetrated by plant roots and percolating water. The upper few inches of the subsoil consists of distinctly heavier, mottled, deep-red and brown, medium-compact clay, which slightly hinders the percolation of water and interferes with good development of roots. At a depth of about 14 inches, the soil material becomes mottled gray, yellow, and red, acid clay, having about the same physical characteristics as the layer above. The material continues to a depth of more than 5 feet and becomes slightly less compact with depth, but is distinctly mottled. Small angular fragments of shale, some of which are in different stages of weathering, are scattered over the surface and throughout the soil in various quantities.

[1] Respectively, Head Dept. of Soils, Assoc. Soil Conservationist, and two Jr. Soil Conservationists. This work was undertaken in cooperation with the Soil Conservation Service of Puerto Rico, of which Mr. E. A. Telford is Associate Soil Conservationist, and the Federal Experiment Station at Mayaguez.

"Catalina clay" is one of the most extensive all-round farming soils in the humid uplands. The relief ranges from rolling to steep, but most of the hills are rounded and gently sloping ravines intervene. It is an acid soil, lateritic, derived from andesitic tuff and tuffaceous shale. A cultivated soil ranges in color from red to light brown. Few of the large fields are uniform in color, because deep plowing and sheet erosion have exposed the red subsoil that lies below the original brown or reddish-brown surface soil, and the result is a heterogeneous color. Normally, the surface soil is a light reddish-brown, friable, softly granular clay that forms large clods when plowed; but which soon slakes into fine granules after the first or second dashing tropical shower. The thickness of the surface soil, as well as the value of the land, varies with the relief. On the less valuable, steeper, more eroded slopes, the surface soil does not average more than 4 inches in thickness,

TABLE 1

Distances of planting of the leguminous crops

Crops	Leguminosae	Between rows	Between plants
			inches
First	Velvet beans	3 feet	18
	Crotalaria	14 inches	Continuous
	Cowpeas	3 feet	4
	Soybeans	2.5 feet	4
Second	Velvet beans	1 foot	6
	Crotalaria	1 foot	Continuous
	Cowpeas	1 foot	4
	Soybeans	1 foot	4

and when the land is plowed some of the subsoil is exposed. On the more rolling, more valuable, less eroded areas, the surface soil is generally about 8 inches thick. The upper part of the subsoil, ranging from a depth of 8 to 24 inches, is brownish-red or red heavy, but friable, slightly-granular clay. Both the surface soil and the subsoil are penetrated readily by plant roots and percolating water; and as a consequence, sheet erosion is less severe than on soils with more plastic subsoil, which absorb water more slowly. The lower part of the subsoil, beginning at a depth of about 24 inches, is a dark-red clay that is more friable than the material in the layers above. The uniform red clay continues to a depth ranging from 10 to 30 feet, before the parent rock is reached. Areas of "Catalina clay" in which the relief ranges from nearly level to slightly undulating, are classified as "Catalina clay", level phase. It has not been affected by sheet erosion, and the normal soil-forming processes have been acting unmolested on it for a long time.

TABLE 2

Dates of planting and harvesting and age of crops

Crops	Location	Leguminosae	Date of planting	Date of harvesting	Age
			1944	*1944*	*days*
First	Río Piedras	Velvet beans	June 21	Sept. 6	77
		Crotalaria	8	Aug. 14	67
		Cowpeas	26	3	38
		Soybeans	21	16	56
Second	Río Piedras	Velvet beans	Oct. 6	Dec. 7	62
		Crotalaria	26	11	46
		Cowpeas	16	6	51
		Soybeans	20	5	46
First	Mayaguez	Velvet beans	May 29	Sept. 5	103
		Crotalaria	30	Aug. 4	66
		Cowpeas	29	July 18	50
		Soybeans	30	26	57
Second	Mayaguez	Velvet beans	Sept. 21	Nov. 23	63
		Crotalaria	Oct. 16	poor growth	
		Cowpeas	Sept. 26	Nov. 20	55
		Soybeans	Oct. 9	Nov. 21	43

TABLE 3

Yields per acre of two consecutive crops of leguminosae in the absence or presence of lime and phosphoric acid

Treatment	Rio Piedras				Mayaguez			
	Field "A"		Field "B"		Field "A"		Field "B"	
	First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop
	green weight				*green weight*			
	cwt.	*cwt.*	*cwt.*	*cwt.*	*cwt.*	*cwt.*	*cwt.*	*cwt.*
Velvet beans	93.1	89.6	126.9	111.4	94.8	63.0	54.2	34.7
Velvet beans with lime & P_2O_5	125.6	113.3	135.3	133.0	126.3	98.9	86.8	60.8
Crotalaria	33.0	6.8	84.4	10.1	29.1		6.5	
Crotalaria with lime & P_2O_5	41.3	10.3	117.3	7.4	31.2		6.1	
Cowpeas	96.2	97.0	69.9	92.3	81.7	60.7	51.9	24.4
Cowpeas with lime & P_2O_5	96.0	106.2	94.8	122.8	89.3	72.1	67.5	30.3
Soybeans	16.6	25.1	38.9	30.7	43.2	22.4	22.6	12.6
Soybeans with lime & P_2O_5	24.9	40.2	53.9	48.7	88.0	35.6	41.5	16.4
	dry weight				*dry weight*			
	cwt.	*cwt.*	*cwt.*	*cwt.*	*cwt.*	*cwt.*	*cwt.*	*cwt.*
Velvet beans	20.4	18.2	27.0	22.2	17.2	12.6	12.7	9.1
Velvet beans with lime & P_2O_5	26.8	22.9	28.5	24.6	22.4	19.9	19.4	16.2
Crotalaria	4.7	1.2	11.9	1.6	5.0		1.5	
Crotalaria with lime & P_2O_5	5.9	2.3	16.2	1.2	5.3		1.4	
Cowpeas	9.4	13.4	7.1	13.0	12.3	10.7	9.2	5.4
Cowpeas with lime & P_2O_5	9.7	14.9	9.3	17.1	13.5	13.1	12.2	6.8
Soybeans	2.4	4.5	5.2	5.8	9.1	5.1	6.1	3.7
Soybeans with lime & P_2O_5	3.8	7.4	7.6	9.2	18.7	8.5	9.8	5.1

TABLE 4

Mean dry-weight yields per acre of all crops in Río Piedras and Mayaguez, excluding Crotalaria

Leguminosae	Treatment	General
	cwt.	*cwt.*
Velvet beans, no lime & phosphorus	17.42	
Velvet beans lime & phosphorus	22.58	
Velvet beans		20.00
Cowpeas no lime & phosphorus	10.05	
Cowpeas, lime & phosphorus	12.07	
Cowpeas		11.06
Soybeans, no lime & phosphorus	5.25	
Soybeans, lime & phosphorus	8.76	
Soybeans		7.01

Values to be exceeded for significance by difference between means of:

Treatments	General
At 5% point; $1.967\sqrt{\frac{2 \times 20.42}{72}} = 1.48$	$1.967\sqrt{\frac{2 \times 20.42}{144}} = 1.05$
At 1% point; $2.590\sqrt{\frac{2 \times 20.42}{72}} = 1.95$	$2.590\sqrt{\frac{2 \times 20.42}{144}} = 1.38$

TABLE 5

Mean dry-weight yields per acre of all crops in Río Piedras

Leguminosae	Treatment	General
	cwt.	*cwt.*
Velvet beans, no lime & phosphorus	22.10	
Velvet beans, lime & phosphorus	25.54	
Velvet beans		23.82
Cowpeas, no lime & phosphorus	11.86	
Cowpeas, lime & phosphorus	11.59	
Cowpeas		11.73
Soybeans, no lime & phosphorus	4.53	
Soybeans, lime & phosphorus	6.96	
Soybeans		5.74
Crotalaria, no lime & phosphorus	3.53	
Crotalaria, lime and phosphorus	7.74	
Crotalaria		5.63

Values to be exceeded for significance by difference between means of:

Treatments	General
At 5% point; $1.971\sqrt{\frac{2 \times 2.75}{36}} = 0.77$	$1.971\sqrt{\frac{2 \times 2.75}{72}} = 0.54$
At 1% point; $2.599\sqrt{\frac{2 \times 2.75}{36}} = 1.02$	$2.599\sqrt{\frac{2 \times 2.75}{72}} = 0.72$

It differs from the typical soil, in that it has a thicker surface soil and a slightly more compact, lower subsoil layers; causing slightly restricted internal drainage which is reflected by the mottled gray, reddish-brown, and red coloring of the normally red lower subsoil layer.

TABLE 6

Analysis of the total sum of squared deviations, of the dry-weight for all crops of Leguminosae at Río Piedras and Mayaguez, excluding the Crotalaria

Source	Degrees of freedom	Sum of squares	Variance	F
Zone	1	499.66	499.66	16.14*
Field	1	117.19	117.19	3.79
Zone x Field	1	910.02	910.02	29.40*
Crop	1	177.36	177.36	5.73*
Crop x Field	1	.45	.45	
Crop x Zone	1	732.16	732.16	
Crop x Zone x Field	1	.53	.53	
Error (a)	64	1980.64	30.95	
Treatments	5	14276.68	2855.34	39.83*
Species	2	12731.07	636.55	31.17*
Fertilizer	1	1368.89	1368.89	67.04*
Species x Fertilizer	2	176.72	88.36	3.05
Treatment x Field	5	131.72	26.34	
Species x Field	2	72.63	36.31	
Fertilizer x Field	1	9.54	9.54	
Species x Fertilizer Field	2	49.55	24.78	
Treatments x Crop	5	497.91	99.58	4.88*
Species x Crop	2	473.77	236.88	11.60*
Fertilizer x Crop	1	.50	.50	
Species x Fertilizer x Crop	2	23.64	11.82	
Treatments x Crop x Field	5	22.00	4.40	
Species x Crop x Field	2	13.07	6.54	
Fertilizer x Crop x Field	1	1.01	1.01	
Species x Fertilizer x Crop x Field	2	7.92	3.96	
Total SS	431	26288.50		
		−19346.32		
Error (b)	340	6942.18	20.42	

* Significant at the 1% point.

Each one of the four experimental fields was divided into 72 plots, three with plots having an approximate area of one-hundredth acre; and one at Mayaguez, with plots having an approximate area of one-hundred twentieth

acre. Half of the plots in each field received limestone at the rate of four tons per acre, and superphosphate at the rate of one-hundred pounds P_2O_5 per acre. Velvet beans *Stizolobium deeringianum*; crotalaria *Crotalaria striata*; cowpeas *Vigna sinensis*; and soybeans *Soja max*, Otootan variety;

TABLE 7

Analysis of the total sum of squared deviations of the dry-weight data for all four crops of Leguminosae at Río Piedras

Source	Degrees of freedom	Sum of squares	Variance	F
Field	1	431.69	431.69	17.04†
Crop	1	76.06	76.06	3.00
Crop x Field	1	109.51	109.51	4.32*
Error (a)	32	811.02	25.34	
Treatments	7	16270.61	2324.37	845.22†
Species	3	15786.11	5262.04	1754.00†
Fertilizer	1	438.08	438.08	146.00
Species x Fertilizer	3	46.42	15.47	5.63†
Treatment x Field	7	292.01	41.72	15.17†
Species x Field	3	206.47	68.82	25.03†
Fertilizer x Field	1	0.00		
Species x Fertilizer x Field	3	85.54	28.51	10.37†
Treatment x Crop	7	2049.87	292.84	106.49†
Species x Crop	3	2005.91	668.64	243.14†
Fertilizer x Crop	1	0.00		
Species x Fertilizer x Crop	3	43.96	14.65	
Treatment x Crop x Field	7	2786.21	398.03	144.74†
Species x Crop x Field	3	2619.80	873.27	317.55†
Fertilizer x Crop x Field	1	110.23	110.23	40.08†
Species x Fertilizer x Crop x Field	3	56.18	18.73	6.81†
Total SS	287	23,442.18		
		−22,826.98		
Error (b)	224	615.20	2.75	

* Significant at the 5% point.
† Significant at the 1% point.

were selected for the Leguminosae. The seeds of soybeans at Río Piedras were inoculated with commercial Nitragin culture. All the other seeds were inoculated by the soil-paste or muddy-water method. Each treatment was replicated nine times.

Two consecutive crops of each one of the Leguminosae, planted at convenient distances (table 1) were harvested from each field (table 2). The

first crops, except the velvet beans, were harvested at the preblooming period. The second crops were harvested at approximately the same dates.

Green-weight and dry-weight yields, for each one of the two crops of Leguminosae at Río Piedras and Mayaguez, are reported in table 3. Mean dryweight yields of all crops in Río Piedras and Mayaguez, excluding the crotalaria because of its poor growth in Mayaguez, are reported in table 4. Mean dry-weight yields of all crops in Río Piedras are reported in table 5.

Tables 6 and 7 contain, respectively, the analysis of the total sum of squared deviations of the mean dry weights.

MAJOR RESULTS

In general, the combined experiments at Río Piedras and Mayaguez, excluding the crotalaria (tables 4, 6) indicate that:

1—The mean dry-matter yield of velvet beans in the presence or absence of the lime and phosphorus application or as a whole, was significantly higher at the 1 per cent point, than that of cowpeas and soybeans; whereas that of cowpeas was higher than that of soybeans.

2—The mean dry-matter yield of velvet beans with lime and phosphorus, was significantly higher at the 1 per cent point, than that of velvet beans without lime and phosphorus. The same applies to soybeans, and cowpeas.

In general, the combined experiments at Río Piedras, including the crotalaria (tables 5, 7) indicate that:

1—The mean dry-matter yield of velvet beans in the presence or absence of lime and phosphorus application or as a whole, was significantly higher at the 1 per cent point, than that of cowpeas, soybeans, and crotalaria; whereas that of cowpeas was higher than that of soybeans, and crotalaria. The soybeans was significantly higher at the 5 per cent point, than the crotalaria, in the absence of lime and phosphorus, and significantly lower in their presence; but as a whole there was no significant mean difference between them.

2—The velvet beans, soybeans, and crotalaria, responded significantly at the 1 per cent point, to the application of lime and phosphorus. The cowpeas showed no response to the fertilizer.

SUMMARY

The effect of lime and phosphorus on the yields of velvet beans, crotalaria, cowpeas, and soybeans, is presented for eight crops harvested in the acid soil types Fajardo Clay and Catalina Clay, of Puerto Rico.

Data for distances of plantings, age of crops, green and dry-weight yields for each of two consecutive crops of the leguminosae in each of four fields, and for the mean dry-weight yields of all crops are presented. Analysis for the total sum of squared deviations of the dry weight for the various crops, is also presented and discussed.

Velvet beans, the highest yielder, gave, in the absence or presence of lime and phosphorus, mean green weights of 83.5 and 110.0 hundredweights per acre, respectively, equivalent to mean dry weights of 17.4 and 22.6 hundred weights per acre.

RESUMEN

Se expone aquí el efecto de la cal y el fósforo en relacion con el rendimiento de las habichuelas "terciopelo", crotalarias, fréjoles y habas sojas de ocho cosechas, producidas en los tipos de suelos ácidos "Fajardo arcilloso" y "Catalina arcilloso" de Puerto Rico.

Se hace una exposición sobre la distancia de siembra, edad de las cosechas, rendimientos de dos cosechas consecutivas a base de peso verde y peso seco de las leguminosas de cada una de las cuatro fincas, así como del promedio de peso seco de todas las cosechas. Se expone y discute también el análisis de la suma total de las desviaciones cuadráticas del peso de las diversas cosechas a base de peso seco.

Las habichuelas "terciopelo", que fueron las que dieron mayor rendimiento, produjeron, en ausencia o presencia de la cal y el fósforo, un promedio de peso verde de 83.5 y 110.0 quintales por acre, respectivamente; equivalente a un promedio de peso seco de 17.4 y 22.6 quintales por acre.

FIG. 1. General view of the legume plots as laid out on the contour at Mayaguez

FIG. 2. Two adjacent soybean plots at Mayaguez. The plot at the left received lime and phosphorus

FIG. 3. Plot in foreground is one of velvet beans with lime and phosphorus, at Río Piedras

FIG. 4. General view of field at Río Piedras. Crotalaria without lime and phosphorus in foreground and center. Velvet beans with lime and phosphorus in-between

FUNGI OF THE DOMINICAN REPUBLIC—XYLARIACEA

By JULIAN H. MILLER[1]

The Xylariaceae of the Dominican Republic are relatively little known, and in Ciferri's list (2), only thirteen species are reported. In Puerto Rico and the Virgin Islands, Seaver and Chardón (4), report fifty-three species from a much smaller territory. Our unsatisfactory knowledge of the Dominican Xylariaceae is due to the fact that previous mycological explorers showed little or no interest in collecting saprophytic forms, confining themselves almost exclusively to plant parasites.

M. J. Berkeley (1) in 1852, reported the following species:

1. **Hypoxylon concentricum** Berk
   Now *Daldinia concentrica* (Bolt. ex Fr.) Ces. & De Not.
2. **Hypoxylon comosum** Berk
   Now *Xylaria comosa* (Berk.) Mont.
3. **Hypoxylon domingensis** Berk
   Now *Xylaria domingensis* (Berk.) Sacc.
4. **Hypoxylon grammicum** Berk
   Now *Xylaria grammica* (Berk.) Mont.
5. **Hypoxylon obtusissima** Berk
   Now *Xylaria obtusissima* (Berk.) Sacc.

Feé (3) in 1835, was the first to report a species of this group from Santo Domingo, describing *Sphaeria divaricata*, which is now called *Xylaria divaricata* (Feé) Sacc.

R. A. Toro (5), in 1927, reported six additional species, one of which, *Daldinia concentrica*, had already been collected. The rest of them were:

6. **Kretzschmaria rugosa** Earle
7. **Nummularia Bulliardi** Tul
8. **Xylaria apiculata** Cooke
9. **Xylaria arbuscula** Sacc
10. **Xylaria consociata** Starb

With regards to Toro's collections, the following comments are pertinent: *Kretzschmaria rugosa* is a synonym of *Xylaria anisopleura*, *Nummularia Bulliardi* apparently does not occur in the tropics, and *Xylaria apiculata* is not in America. His number *315*, reported as *Xylaria consociata*, has been examined and found to be *Xyl. Hypoxylon;* and his number *320*, reported as *Xylaria apiculata*, has been included under the present study and found to be *Xyl. multiplex*.

In view of the above confusing records, and the fact that Berkeley's collection has not been examined, it is difficult to determine the number

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of valid species known from the flora. Provisionally, this may be figured at 9, that is Berkeley, 5; Feé, 1; and Toro, 3.

The present study of the Xylariaceae of the Dominican Republic has been based on collections made by Dr. Carlos E. Chardón, during the summer of 1937. It contains twenty-four species, none of which were previously reported, and are consequently new to the flora. Duplicates of all specimens have been kept by the writer.

Hypoxylon Bull., Champ. Fr. **1:** 168. 1791.

1. **Hypoxylon deustum** (Hoffm ex Fr)
Grev., Scott. Crypt. Fl. **6:** 324. 1828.
Sphaeria maxima Hall
Hist. Stirp. Helv. **3:** 122. 1768.
Sph. deusta Hoffm
Veg. Crypt. **1:** 3. 1787.
Sph. versipellis Tode
Fung. Meckl. Sel. fasc. **2:** 55. 1791.
Hyp. ustulatum Bull
Champ. Fr. **1:** 176. 1791.
Sph. deusta Hoffm ex Fr
Syst. Myc. **2:** 345. 1823.
Hyp. ustulatum Bull ex Fr
Summa Veg. Scand. p. 383. 1849.
Ustulina vulgaris Tul
Sel. Fung. Carp. **2:** 23. 1863.
Ust. deusta (Hoffm ex Fr)
Petrak, Ann. Myc. **19:** 279. 1921.
Nemania maxima (Hall) House
N. Y. State Mus. Rep. **266:** 48. 1925.

A closely related form, *Ustulina zonata* (Levé.) Sacc., is usually considered distinct due to wider and more rounded spores, but the writer does not think the difference is sufficient to warrant a specific distinction. This form, rather than *Hyp. deustum*, is the usual one in the tropics.

On dead wood.

La Vega: Forests at El Hatillo, *Chardón 1092*, August 28, 1937.

2. **Hypoxylon fossulatum** Mont
Crypt. Guyan., Ann. Sci. Nat. IV, **3:** 123. 1855.
Nummularia fossulata (Mont) Cke
Grev. **11:** 127. 1883.

This is an interesting fungus in that it is an annulate form, closely related to *Hyp. Stygium* (Levé.) Sacc., but is constantly smooth, not show-

ing the perithecial elevations. The asco-spores are 6–8 x 2.5μ. The type is Leprieur *692* from Cayenne, but the writer has specimens from Trinidad and Puerto Rico as well as many other countries.

On dead wood.

La Vega: Ravine near Jarabacoa, 550 m., *Chardón 949*, Aug. 5, 1937.

3. **Hypoxylon glomeratum** Cke
Grev. **11:** 134. 1883.
Hyp. Mascariensis Berk ex Cke
Grev. **11:** 131. 1883. Non Mont.
Hyp. anthracoderma Speg
Fung. Guar. Pug. **3:** 28. 1888.
Hyp. Berkeleyi Sacc
Syll. Fung. **9:** 551. 1891.
Hyp. Bakeri Earle
Bull. Torr. Bot. Club **26:** 633. 1899.
Hyp. vinosa-purpureum Ell & Ev
Fl. Lud. Nom. nud.
Hyp. cohaerens Pers ex Fr var. *brasiliensis* Starb
Ascom. Reg. Exp. **2:** 8. 1901.
Hyp. rubigineo-areolatum Rehm var. *microspora* Theiss
Ann. Myc. **6:** 345. 1908.
Hyp. Merrillii Syd
Ann. Myc. **15:** 212. 1917.
Hyp. cupricolor Petch
Ann. Roy. Gard. Peradeniya **8:** 158. 1924.
Hyp. rubigineo-areolatum Rehm var. Bakeri (Earle) Mill
Mycol. Explor. Colombia, Jour. Dept. Agri. P. R. **14:** 273. 1930.

This species is widely spread over both North and South America.

On dead wood.

La Vega: Trail from Maimón to Yuna River, Chardon 1028, Aug. 13, 1937; Forest at El Hatillo, *Chardón* 1086 & 1095, Aug. 28, 1937.

4. **Hypoxylon sclerophaeum** Berk & Curt
Exot. Fung. Schw., Jour. Acad. Nat. Sci. Phila. 2nd. ser. **2:** 285. 1853.
? *Sphaeria coelata* Fr
Linnaea **5:** 540. 1830.
Hyp. placentiforme Berk & Curt
Cuban F., Jour. Linn. Soc. **10:** 383. 1869.
Hyp. Wrightii Berk & Curt
l. c. 1869

Hyp. coelatum Ces
Fung. Born. Atta. Acc. Sci. fisch e matim. d Napoli **8:** 19. 1879.
Nummularia Wrightii (Berk & Curt) Sacc
Syll. F. **1:** 398. 1882.
Numm. suborbicularis (Berk & Curt) Sacc var. *Cookeana* Sacc
Syll. Fung. **1:** 399. 1882.
Numm. placentiformis (Berk & Curt) Sacc
Syll. Fung. **1:** 399. 1882.
Hyp. nicaraguense Ell & Ev
Iowa Univ. Bull. **2:** 394–415. 1893.
Penzigia polyporus Starb
Ascom. der Schwed. Chaco-Cordill. Exped. p. **32,** Ark. fur Bot. **5.** 1905.
Daldinia placentiformis (Berk & Curt) Theiss
Ann. Myc. **7: 4.** 1909.
Numm. Cookeana (Sacc)
Rehm, Ascom. Phil. Leafl. Bot. **6:** 2273. 1914.
Pyrenopolyporus Hunteri Lloyd
Myc. Notes **49:** 705, f. 1054. 1917.
Hyp. amorphum Ell & Ev
Nom. nud. Kew herb.
Hypodiscus placentiformis (Berk & Curt)
Rick, Broteria ser. Bot. **25:** 34. 1931.

On fence posts.

La Vega: Maimón River, near Maimón, *Chardón* 1017, Aug. 13, 1937

5. **Hypoxylon serpens** Pers ex Fr
Summa Veg. Scand. p. 384. 1849.
Sphaeria macula Tode
Fung. Meckl. **2:** 33. 1791.
Sph. serpens Pers
Syn. Meth. F. p. 20. 1801.
Sph. uda Pers
Syn. Meth. F. p. 33. 1801.
Sph. colliculosa Schw
Syn. Car no. 82. 1822
Sph. serpens Pers ex Fr
Syst. Myc. **2:** 341. 1823.
?*Sph. unita* Fr
Elench. Fung. **2:** 67. 1828.
Sph. caries Schw
Trans. Amer. Phil. Soc., Phila. n. ser. **4:** 194. 1832.

Hyp. bipapillatum Berk & Curt
Exot. Fung. Schw., Journ. Phila. Acad. Sci. II. **2:** 285. 1853.
Sph. capnodes Berk & Br
Hooker Lond. Jour. **4:** 72. 1845.
Hyp. colliculosum (Schw) Curt
Geol. & Nat. Hist. Surv. N. C., pt. III, p. 140. 1867.
Hyp. unitum (Fr) Nits
Pyren. Germ. p. 44. 1867.
Hyp. aeneum Nits
Pyren. Germ. p. 47. 1867.
Hyp. effusum Nits
Pyren. Germ. p. 48. 1867.
Hyp. reticulatum Karst
Not ur Sallsk. pro Fauna et Fl. Fenn. II, p. 238. 1871–74.
Hyp. allantoideum Cke
Grev. **8:** 66. 1879.
Hyp. caries (Schw) Sacc
Syll. Fung. **1:** 393. 1882.
Anthostoma capnodes (Berk & Br) Sacc
Syll. Fung. **1:** 298. 1882.
Hyp. ramosum Schw ex Cke
Grev. **11:** 132. 1883.
Hyp. irregulare Cke
Grev. **11:** 133. 1883.
Ustulina linearis Rehm
Hedw. **33:** 310. 1892.

This species has the same general appearance as the mature stage of *Hyp. glomeratum*. The distinction lies in the spore measurements. The ascospores of the former are usually between 10 and 15μ. long, while those of the latter are from 9 to 11μ. Also in the early stages *Hyp. serpens* is dirty white, easily distinguishing it from *Hyp. glomeratum* which is a bright red.

Hyp. serpens is world wide in distribution, but is rarely found in the tropics.

On dead wood.

La Vega: Forests at El Hatillo, *Chardón 1090* Aug. 28, 1937.

6. **Hypoxylon Stygium** (Lév) Sacc
Syll. Fung. **1:** 379. 1882.
Sphaeria Stygia Lév
Ann. Sci. Nat. **5:** 258. 1846.
Hyp. Puiggarii Speg

Fung. Puigg. Pug. I., Bol. Acad. Cien. Cordoba II, n. 257. 1889.
Hyp. annuliforme Rehm
Verh. bot. Brand. p. 65. 1890.
Hyp. platystomum Ell & Ev
N. Amer. Pyr. p. 649. 1892.
Hyp. microcarpum Penz & Sacc
Malphigia **11:** 492. 1897.
Hyp. Bogariense v. Höhn
Stzb. K. Akad. d. Wiss. Wien. 118, abt. I, p. 341. 1909.
Hyp. punctatum Petch
Ann. Roy. Bot. Gard, Peradeniya **8:** 153. 1924.

The number *763* shows very prominent perithecia, while in number *1022* the stroma is plane as in forms previously placed in *Nummularia*.

The type was collected from St. Domingue by Poiteau, and is in the herbarium of the Museum of Paris. No. *1022* is similar to the type.

On dead branches of tree.

Azua: Las Caobas ravine, south of San José de Ocoa, *Chardón 763*, July 10, 1937.

La Vega: Trail from Maimón to Yuna River, *Chardón 1022*, Aug. 13, 1937.

7. **Hypoxylon tinctor** (Berk) Cke
Grev. **11:** 135. 1883.
?*Sphaeria clypeus* Schw
Syn. Car. n. 42. 1822.
Sph. tinctor Berk
Hook. Lond. Journ. Bot. **4:** 311. 1845.
Hyp. clypeus Schw ex Curt
Geol. & Nat. Hist. Surv. N. C. III, p. 140. 1867.
Diatrype clypeus Schw ex Berk
Grev. **4:** 95. 1876.
Diatrype tinctor (Berk) Sacc
Syll. Fung. **1:** 200. 1882.
Hyp. applanatum Cke
In herb. Kew. Nom. nud.
Nummularia clypeus Schw ex Cke
Grev. **12:** 6. 1883. Excl. Cke. Spec.
Numm. tinctor (Berk.) Ell & Ev
N. Amer. Pyr. p. 627. 1892.

Many specimens of this species, especially from the tropics, do not exhibit the discolored substratum, but No. 627 shows this orange color in just as marked a manner as in specimens from the southern United States.

On dead wood and dead trunk of *Ficus nitida* (No. *627*)

Azua: River forest, near San Juan de la Maguana, *Chardón 627*, June 27, 1927; Public Square, San José de Ocoa, *Chardón 761*, July 9, 1937.

Kretzschmaria Fries, Summa Veg. Scand. p. 409. 1849.

8. **Kretzschmaria cetrarioides** (Curr & Welw) Sacc., Syll. Fung. **9:** 966. 1891.
Hypoxylon cetrarioides Curr & Welw
Trans. Linn. Soc. **26:** 282. 1869.
Sphaeria lichenoides Berk
Kew herb. Nom. nud.
Rhopalopsis lichenoides (Berk) Cke
Grev. **11:** 94. 1883.
Kretz. lichenoides (Berk) Sacc
Syll. F. **2:** Add. ad vol. 1, XXIX. 1883.
Rhopalopsis cetrarioides (Curr & Welw) Cke
Grev. **11:** 127. 1883.
Kretz. lichenoides Rick
Ann. Myc. **7:** 8. 1909.
On dead wood.
La Vega: Forests road to Cotui, *Chardón 1077*, August 6, 1937.

9. **Kretzschmaria clavus** Fr. Summa Veg. Scand. p. 409. 1849.
On dead wood.
La Vega: Ravine, km. 67, road to Bonao, *Chardón 800*, July 18, 1937; Forests road to Cotui, *Chardón 1077*, Aug. 6, 1937; Forests at El Hatillo, *Chardón 1091*, *1093*, Aug. 28, 1937.

Nummularia Tul., Fung. Carp. **2:** 42. 1863.

10. **Nummularia artocreas** (Cke & Mass) comb. nov.
?Hypoxylon labellum Mont
Ann. Sci. Nat. IV, **3:** 511, t. 5, fig. 5. 1855.
?Camillea labellum Mont
Syll. Crypt. p. 208. 1856.
Diatrype artocreas Cke & Mass
Grev. **21:** 4. 1892.
Numm. repanda Fr var. *zonata* Ell & Ev
Iowa Univ. Bull. **2:** 394–415. 1893.
Nummularoidea artocreas (Cke & Mass) Lloyd
Myc. Notes **7:** 1281, f. 2872. 1924.
Camillea artocreas (Cke & Mass) Rick
Broteria ser. bot. v. XXV, fasc. 1, p. 38. 1931.

Lloyd, l.c. fig. 2871, shows a photograph of *Cam. labellum*, and in fig. 2872, one of *Numm. artocreas*. These are the types and are identical in structure, but the Montagne specimen is sterile while the type of *Numm. artocreas* has ascospores 12–15 x 5μ.

This fungus looks much like *Numm. repanda* (Fr.) Nits., but it is distinct in the presence of much finer ostiola and in the sharply fusoid ascospores. Also, the latter are very faint yellow even in old specimens, but in *Numm. repanda* they are dark brown.

This is the common *Nummularia* in the West Indies and in South America. The type is from St. Vincent (B. W. I.). The writer has specimens from Puerto Rico, Jamaica, Nicaragua and Costa Rica.

On dead wood.

La Vega: Forests road to Cotui, *Chardón 961*, August 6, 1937.

Penzigia Sacc. Myc. Malac. p. 20. 1888.

11. **Penzigia frustulosa** (Berk & Curt) Mill
Myc. Explor. Ven. Univ. P. R. Monogr. ser. B, No. 2: 211. 1934.
Hypoxylon frustulosum Berk & Curt
Cuban F., Jour. Linn. Soc. **10:** 383. 1869.
Hyp. leucocreas Berk & Rav
Grev. **4:** 51. 1875.
Hyp. microsporum Ces
Myc. Born. Atta. Acc. Sci. fisich. e Matim. di Napoli **8:** 17. 1879.
Nummularia frustulosa (Berk & Curt)
Syll. F. **1:** 398. 1882.
Hyp. exiguum Cke
Grev. **11:** 130. 1883.
Xylaria frustulosa (Berk & Curt) Cke
Grev. **12:** 5. 1883.
Hyp. lilliputianum Syd
Ann. Myc. **8:** 37. 1910.
Penzigia microspora Petch
Ann. Roy. Bot. Gard. Peradeniya **10:** 137. 1924.

On dead trunk of tree.

Trujillo: Thickets beyond Bani, *Chardón 592*, June 26, 1937.

Poronia Gledits. Willd. Fl. Berol. p. 400. 1787.

12. **Poronia oedipus** Mont
Syll. Crypt. p. 209. 1856.
Sphaeria (*Poronia*) *punctata* var. *oedipoda* Mont
Ann. Sci. Nat. II, 13: n. 35. 1841.
Sph. incrassata Jungh
Flor. Javae Crypt. p. 87. 1838.

Hyp. oedipus Mont
Cuban Fl. p. 346, t. 13, f. 2. 1838–1842.
Poronia macropoda var. *cladonioides* Ces
In Klotz.-Rab. Herb. Myc. no. 1946.

On horse dung.

Santiago: San José de las Matas, *Chardón 462*, June 6, 1937.

Xylaria Hill., Hist. Pl. p. 72. 1773.

13. **Xylaria arbuscula** Sacc
Michelia **1:** 249. 1878.

On decaying pod of *Hymenea courbaril* L.

La Vega: Forests at El Hatillo, *Chardón 1215*, August 28, 1937.

14. **Xylaria axifera** Mont
Ann. Sci. Nat. IV, **3:** 106. 1855.

On dead wood.

La Vega: Forests at El Hatillo, *Chardón 1098*, August 28, 1937.

15. **Xylaria bulbosa** (Pers ex Fr) Berk & Br
Berk. Out. Brit. Fung. p. 385. 1860.
Sphaeria bulbosa Pers
Obs. Myc. **2:** 63. 1799.
Sph. bulbosa Pers ex Fr
Syst. Myc. **2:** 327. 1823.
Sph. corniformis Fr
Elench. Fung. **2:** 57. 1828.
Xylaria corniformis Fr
Summa Veg. Scand. p. 381. 1849.

This species has been named *Xylaria corniformis* in most North American herbaria. It is black, rugose, without a pellicle and sometimes there is a sterile tip. When growing on a hard substrate there is a bulbose base. The only other species with similar appearance and small spores (8–10 x 4–5μ.) is *Xylaria castorea* Berk., but it differs in being compressed instead of terete.

On dead wood.

La Vega: Forests near Yuna River, El Hatillo, *Chardón 1032*, August 14, 1937; Forests at El Hatillo, *Chardón 1103*, *1106*, August 28, 1937.

16. **Xylaria Chardoniana** (Toro) Mill
Mycol. Expl. Ven. Univ. P. R. Monogr. ser. B., no. **2:** 214. 1934.
Poronia Chardoniana Toro
N. Y. Acad. Sci. Surv. P. R. **8:** 68. 1926.

Heretofore known only from Puerto Rico and Venezuela.

On horse dung.

Santiago: San José de las Matas, *Chardón 456*, June 6, 1937.

17. **Xylaria cristata** Speg

Fung. Argent. **1:** 179. 1880.

On decaying log.

Samaná: Cacao plantations, Sabana de la Mar, *Chardón 730*, July 5, 1937.
La Vega: Forests at El Hatillo, *Chardón 1101*, Aug. 28, 1937.

18. **Xylaria cubensis** Mont

Syll. Crypt. p. 202. 1856.

Hypoxylon cubensis Mont

Ann. Sci. Nat. II, **13:** cent. 2, no. 29. 1840.

Hyp. cubensis Mont

Sagra Fl. de Cuba, p. 347, t. 13, f. 1. 1842.

Xylaria fusca Lloyd

Myc. Writ. **5:** 770, f. 1155, 1156. 1918.

This species is clavate, smooth, fuscous to darker, terete, with small spores, 8–10 x 4–5μ. It is common in South America, the West Indies, and in the southern United States.

On decaying trunk of tree.

Samaná: Cacao plantations, Sabana de la Mar, *Chardón 740*, July 6, 1937.

19. **Xylaria Hypoxylon** (L ex Fr) Grev

Fl. Edin. p. 355. 1824.

Clavaria Hypoxylon L

Sp. Pl. p. 1182. 1753.

Sphaeria Hypoxylon L ex Fr

Syst. Myc. **2:** 327. 1823.

On dead wood.

Trujillo: San Cristobal, *Kern & Toro 315*, Mar. 13, 1937 (reported as *Xylaria consociata* Starb.).
Samaná: Cacao plantations, above Samaná, *Chardón 741*, July 6, 1937.
La Vega: Forests at El Hatillo, *Chardón 1100 & 1102*, Aug. 28, 1937.
Puerto Plata: Forests near Sosúa beach, *Chardón 903*, Aug. 1, 1937.

On dead leaf sheaths of *Roystonea quisqueyana* Bailey.

La Vega: Outskirts of Bonao, *Chardón 899*, July 31, 1937.
Duarte: Near San Francisco de Macoris, *Chardón 1075*, July 18, 1937.

20. **Xylaria inaequalis** Berk & Curt

Jour. Linn. Soc. **10:** 382. 1868.

The surface of the fertile portion is dark brown, and at maturity splits in anastomosing lines in a manner similar to *Xyl. multiplex*, *Xyl. arbuscula* and *Xyl. apiculata*. The spores are 9–11 x 4–5μ. as in *Xyl. multiplex*. It differs in possessing very slender stromata and prominent perithecial elevations.

On decaying trunks.

Samaná: Cacao plantations, Sabana de la Mar, *Chardón 732* & *734*, July 5, 1937.

La Vega: Ravine near Jarabacoa, 550 m., *Chardón 951*, Aug. 5, 1937; Forests at El Hatillo, *Chardón 1083*, Aug. 28, 1937.

On decaying log of *Hura crepitans* L.

Duarte: Road beyond San Francisco de Macoris, *Chardón 806*, July 18, 1937.

21. **Xylaria multiplex** (Kze ex Fr) Berk & Curt
Jour. Linn. Soc. **10:** 381. 1869.
Sphaeria multiplex Kze ex Fr
Linnaea **5:** 536. 1830.

On dead and decaying wood.

Trujillo: San Cristobal, *Kern* & *Toro 320*, Mar. 10, 1926 (reported as *Xylaria apiculata* Cke.).

Samaná: Cacao plantations, Sabana de la Mar, *Chardón 731*, *733* & *979*, July 5, 1937.

La Vega: Ravine, km. 67, road to Bonao, *Chardón* & *Toro 921*, July 18, 1937: Forests at El Hatillo, *Chardón 1097*, *1104*, *1107*, *1221*, Aug. 28, 1937.

22. **Xylaria muscula** Lloyd
Myc. Writ. **6:** 994, pl. 160, f. 1780. 1920.

On dead wood.

La Vega: Forests at El Hatillo, *Chardón 1216*, Aug. 28, 1937.

23. **Xylaria scruposa** (Fr) Berk
Jour. Linn. Soc. **10:** 382. 1869.
Sphaeria scruposa Fr
Elench. Fung. **2:** 55. 1828.
Hypoxylon scruposum (Fr) Mont
Cuban F. p. 350. 1838–1842.

On dead wood.

La Vega: Trail from Maimón to Yuna River, *Chardón 1027*, Aug. 13, 1937; Forests at El Hatillo, *Chardón 1105*, Aug. 28, 1937; La Trinchera, near El Hatillo, *Chardón 1232*, Oct. 31, 1937.

24. **Xylaria tabacina** (Kickx) Berk
Hooker's Jour. Bot. & Kew Misc. **6:** 225. 1854.
Hypoxylon tabacinum Kickx
Bull. Acad. Brux. **8:** 11. 1841.
On decaying log.
La Vega: Ravine, km. 67, road to Bonao, *Chardón* & *Toro 803*, July 18, 1937.

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